GENE2230 Laboratory Techniques

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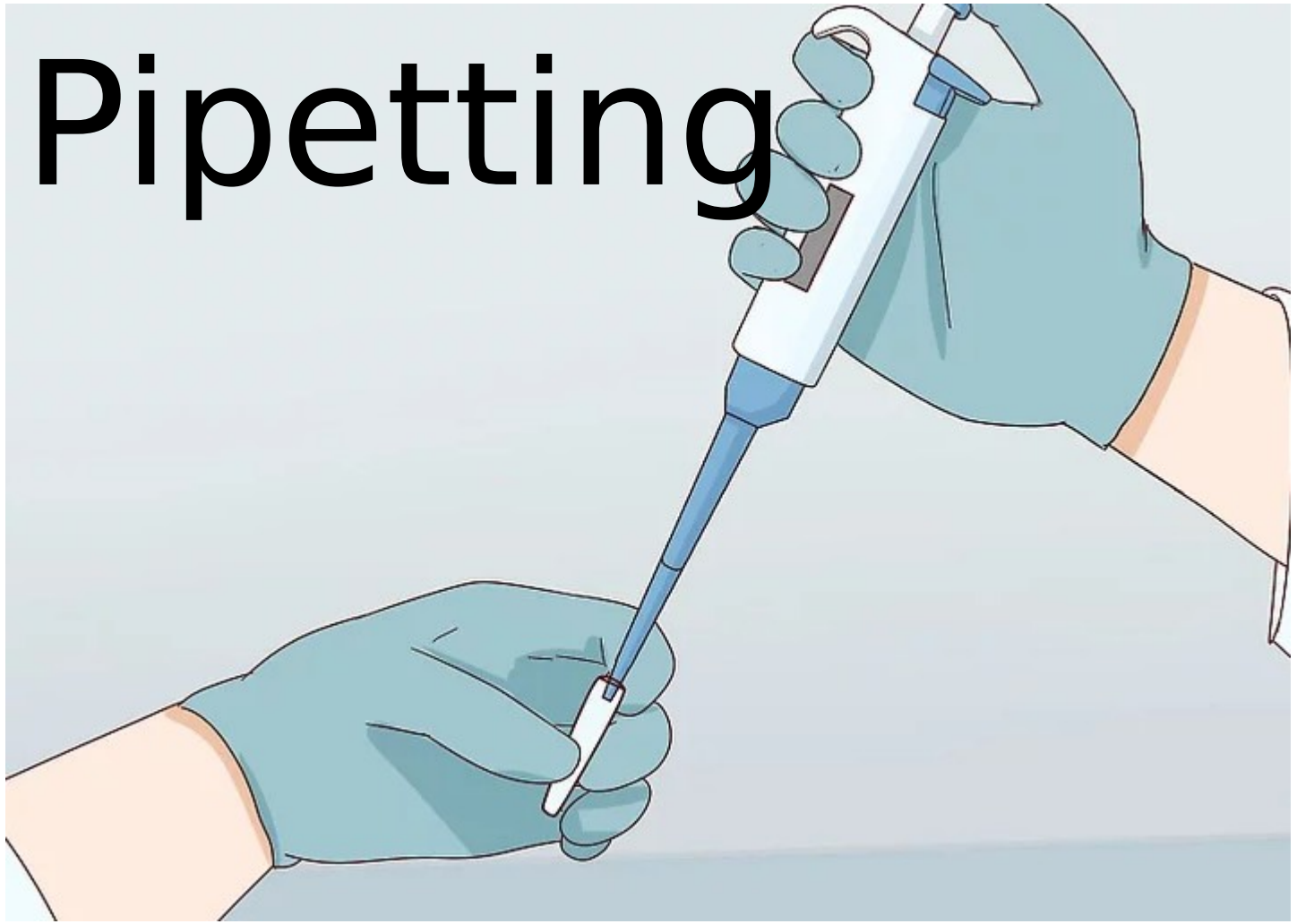
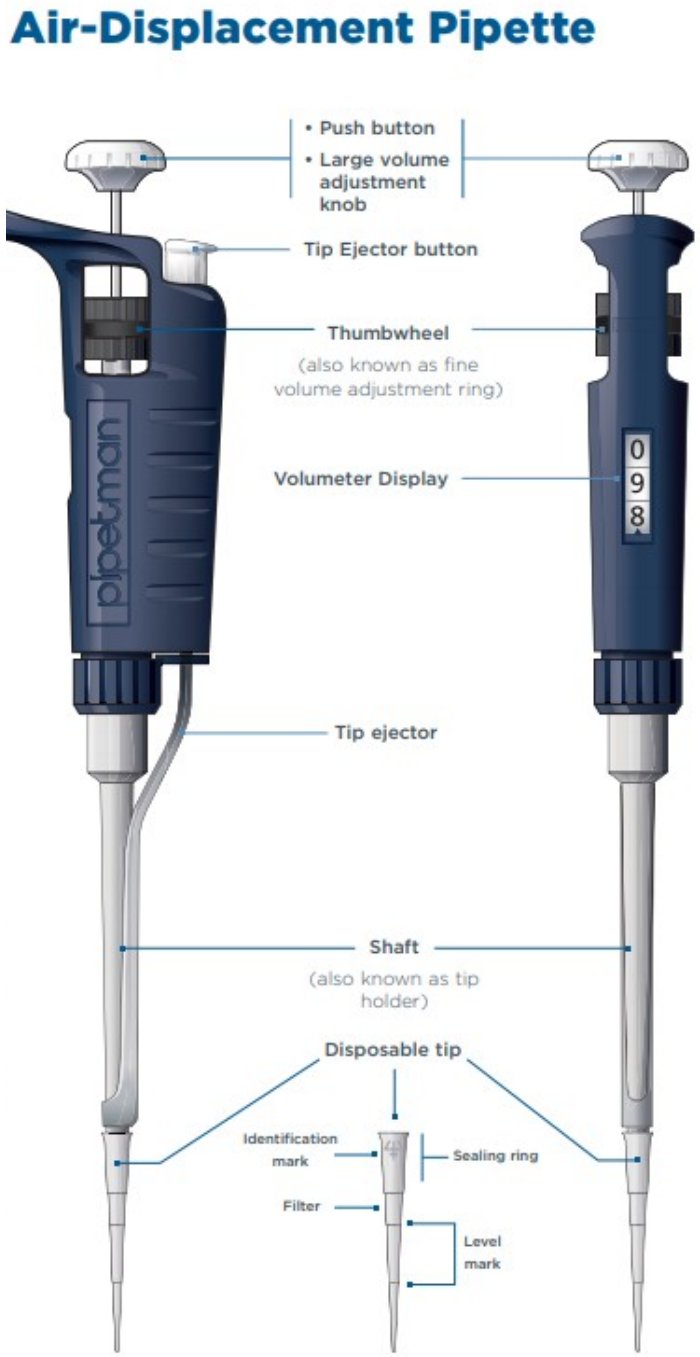
Pipetting

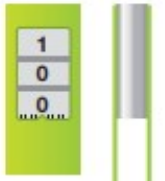


Pipetting

- Gilson micropipettors – used to measure or transfer small amounts of liquids (usually 0.2µl to 1000µl)
- Different pipettes for different volumes – the name indicates max volume (look for labels) – P2 (0.2-2µl), P20 (2-20µl), P200 (20-200µl), P1000 (200-1000µl) - generally select the SMALLEST size for greatest accuracy.
- It is essential that you have good pipetting skills - both accuracy and precision are important.

Using a pipette





1

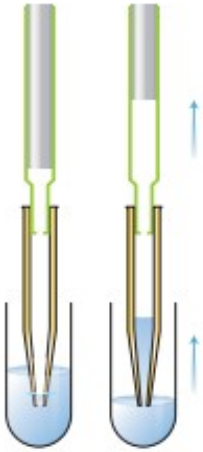
Set Volume

The required volume is set. The piston moves to the appropriate position.

2

Prepare for Aspiration

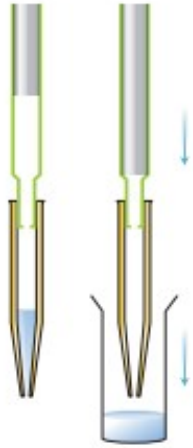
The push button is pressed prior to sample aspiration. The piston descends and expels a volume of air equal to the selected volume of liquid.



3

Aspirate sample

As the push button is released, a partial vacuum is created inside the tip. The ambient atmospheric pressure forces the desired volume of liquid through the orifice into the tip.



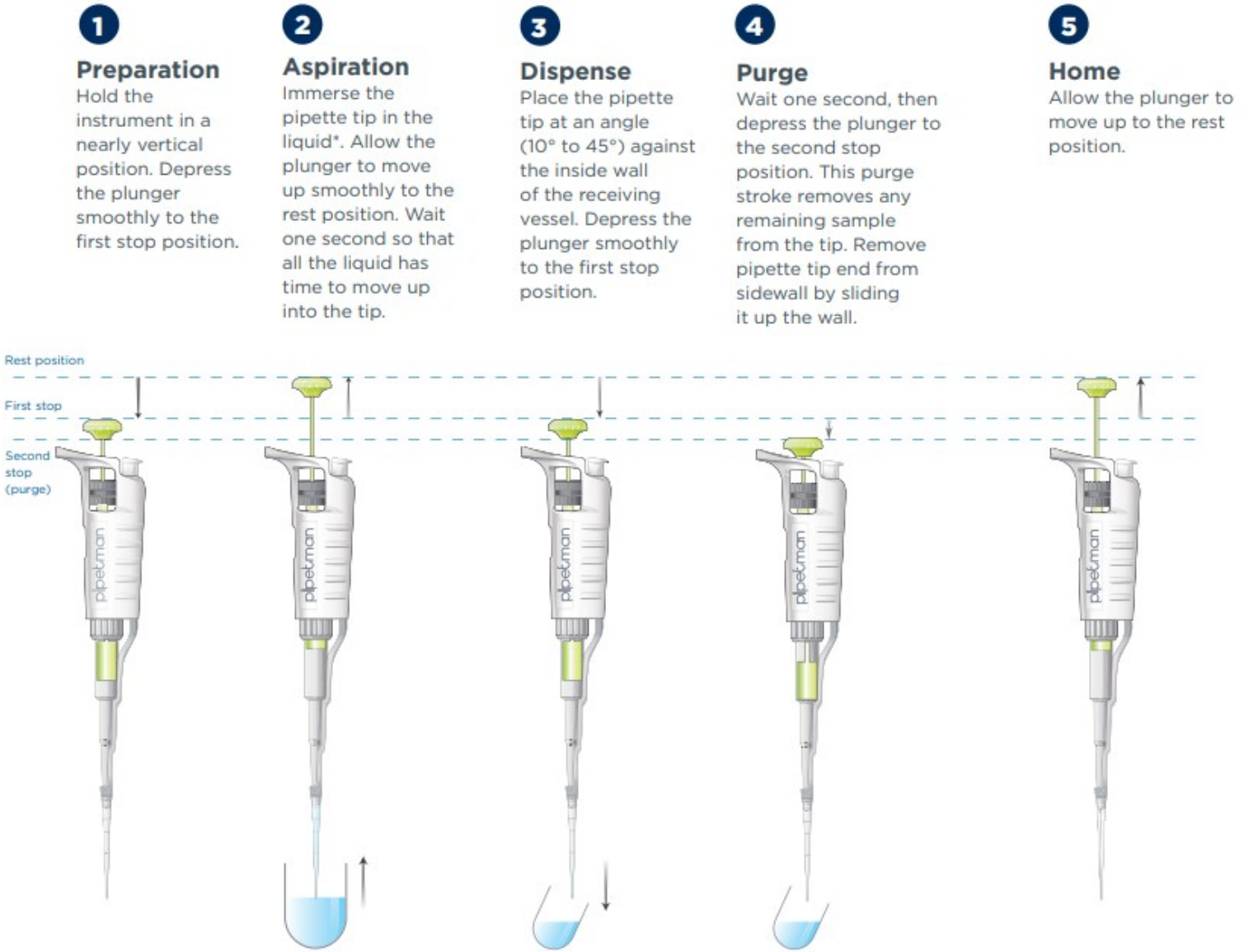
4

Dispense sample

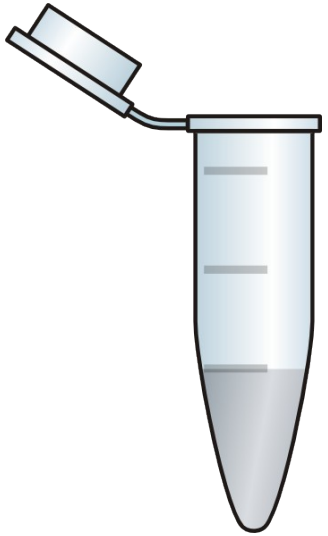
The push button is pressed again. Air pressure increases inside the shaft and the tip. The compressed air pushes the liquid out of the tip.

Accurate pipetting

- Push the pipette onto a tip vertically and firmly
- Immerse the tip *slightly* (a few mm below the meniscus) but consistently when drawing in liquid -- too much will coat the outside of the tip with liquid; too little -> air bubbles.
- Hold pipette vertically when drawing liquid in
- Pipette with a slow, smooth action
- Pause after aspiration before you pull the tip out – liquid keeps flowing after plunger stops
- Pull the pipette straight out (touching the sides of the container from which liquid is drawn will cause wicking and loss of volume)


1
Preparation
Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the first stop position.
2
Aspiration
Immerse the pipette tip in the liquid*. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.
3
Dispense
Place the pipette tip at an angle (10° to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.
4
Purge
Wait one second, then depress the plunger to the second stop position. This purge stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall.
5
Home
Allow the plunger to move up to the rest position.
Rest position
First stop
Second stop (purge)
pipetman

Measuring a volume – ‘wind down’ method



Sample of unknown volume (approx. 100 µl)

1 roughly estimate the volume and select the appropriate pipette. Set the volume to MORE than your estimate (eg, 200 µl)

2 Draw the sample into the tip gently – sample will sit on top of an air pocket

3 "wind-down" the volume on the Gilson by SLOWLY winding the mechanism anti- clockwise until the sample is JUST at the very end of the tip. Now, read the volume on the Gilson

Pipette care

- Store your pipette vertically, using a pipette holder
- Never put your pipette on its side with liquid in the tip
- Dispose of used tips appropriately

Place in order of importance

- Accuracy
- Precision
- Speed
- Contamination
- Health and safety
- Looking after equipment

Online resources

- https://www.gilson.com/pub/static/frontend/Gilson/customtheme/en_US/images/docs/GuideToPipettingE.pdf
- https://www.wikihow.com/Use-an-Eppendorf-Pipette

Videos:

- https://www.youtube.com/watch?v=uEy_NGDfo_8 – I thought this was a great video from University of Leicester – very dramatic!
- https://www.youtube.com/watch?v=QGX490kuKjg – Eppendorf short tutorial on pipetting
- https://www.youtube.com/watch?v=wlO4zJLR8R8 – reading a pipette (the volume)

- 11. Pre-Wetting the Pipette Tip
- When you dispense liquid from your pipette, a coating of the liquid is left on the tip and makes the dispensed volume slightly less than what is displayed on the pipette. Pre-wetting the tip before you pipette will help increase your accuracy. To pre-wet your tip, draw up the liquid into your pipette and then dispense back into the original vessel. The coating is on the tip. Now, when you draw up the liquid again and dispense it into the receiving vessel, none of it will be left on the tip and lost to wetting. This is only recommended for volumes greater than 10 µL.
- 12. Reverse Pipetting
- Use this technique when pipetting viscous liquids or volatile solvents. Reverse pipetting also helps when pipetting ultra-micro samples of 0.5 µL or less. Push the piston down to the purge position (the second stop), then draw the liquid up. There is too much liquid in the tip at this point. However, when the liquid is dispensed by pushing the piston to the aspirate position (the first stop), the extra liquid is left inside the tip. Using this method, the tip is “automatically” pre-wetted. The extra liquid also helps when pipetting volatile solvents, because some of the solvent will tend to evaporate into the air cushion.

- How should pipettes be stored?
  - On their sides
  - Upright in a rack
  - Any way you like

- Laying a pipette on its side with liquid in the tip
  - Is advisable – it will stop the pipette from drying out
  - Should never be done –it will contaminate the pipette